Vol. 3, Nos. 4-6 Apr.—June, 1927 Nos. 31-33

Bulletin of the Agricultural Chemical Society of Japan.

CONTENTS

Published by the
Agricultural Chemical Society of Japan.
C/O Faculty of Agriculture, Tokyo Imperial University.

Single Copy (Postage inclusive) :- ¥ 0.35
Annual Subscription (12 numbers) :- ¥ 3.50

The Agricultural Chemical Society of Japan.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2. No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japaneses texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

DIGESTION AND METABOLISM EXPERIMENTS WITH SHEEP FED ON MIXED HAY.

By Michio Saitoh.

(*From the laboratory of animal nutrition in Miyazaki College of Agriculture.*)

(Received Feb. 5th., 1927.)

Two mature Shropshire sheep of nearly the same age were selected and fed almost half a year on mixed hay as maintenance ration. About thousand kilogrammes of hay were gathered from the uncultivated land, cut 1–2 inches long and mixed thoroughly in order to feed the same quality of hay during experiment. The sheep maintained their body weight every day with certain quantities of this fodder. In the middle period of this experiment, feces and urines excreted by the animals were taken and analyzed.

Detail analytical data of the hay used were already published in Japanese Journal of Zootechnical Science Vol. I. No. 2.

The experimental results here obtained are as follows:

1. Digestibilities of general constituents of this hay do not differ from the results obtained by former investigators. Average digestibilities are:

62 % of dry matter	67 % of organic matter
48 % of crude protein	56 % of crude fat
71 % of carbohydrates	27 % of crude ashes

2. Nitrogen distribution of feces are determined:

Water soluble nitrogen	25 %
Water soluble protein nitrogen	7 %
Nitrogen soluble in 10 % salt solution	7 %
Nitrogen soluble in 75 % alcohol	5 %
Nitrogen soluble in dilute alkali	33 %
Insoluble nitrogen	30 %

3. In carbohydrates of feces, following compounds were determined:

Total carbohydrates in dried feces 48–49%

In hundred percent of total carbohydrates:

Weende's crude fiber	35 %
Cellulose	18–19 %
Cutin	7–9 %
Lignin	7 %
Pentosan	21–24 %
Pentosan-free nitrogen-free extract	45–49 %
Starch and soluble sugars	4 %

4. Feces contained following mineral compounds:

Total crude ashes in dried feces 21–23%

In hundred percent of total ashes :

Potash	2–3 %
Soda	4 %
Lime	9–10 %
Magnesia	4 %
Iron oxide	6–7 %
Phosphoric acid	7–8 %
Chlorine	0.1 %
Sulfuric acid	2 %
Silicic acid and sands	64–67 %

5. As to urines following results were obtained :

Reaction, strongly alkaline

Specific gravity 1.034–1.0571

Dry matter in urine 3.9–6.8

In hundred percent of urinary dry matter :

Organic matter	48 %
Inorganic matter	52 %
Urea	9–15 %
Ammonia (Immediately)	0.2–0.5 %
Hippuric acid	29–38 %
Uric acid	0.3–0.9 %
Creatinin	0.1 %
Other organic matters	2 %

6. In hundred percent of urinary ashes were found following substances :

Potash	56–60 %
Soda	5–6 %
Lime	0.6–0.4 %
Magnesia	3–5 %
Iron oxide	0.4–0.8 %
Phosphoric acid	0.3–0.4 %
Chlorine	24–27 %
Sulfuric acid	7–9 %
Silicic acid	1–4 %

7. Apparent digestibilities were calculated (Average of two sheep) :

Weende's crude fiber	72 %
Weende's nitrogen-free extract	70 %
Cellulose	78 %
Cutin	67 %
Lignin	59 %
Pentosan	72 %
Pentosan free nitrogen extract	68 %

8. After investigation upon nitrogen metabolism, each sheep was found to have digested 7–8 grammes of nitrogen and deposited 2.8–2.9 grammes of nitrogen every day. Sheep excreted every day 12–17 grammes of hippuric

acid i. e. sheep produced every day 3.8–6.5 grammes of glycocoll and 8.8–13.0 grammes of benzoic acid in the body, according to the following formula:

$$C_6H_5CONH\cdot CH_2\cdot COOH \xrightarrow{H_2O} C_6H_5COOH + CH_2(NH_2)\cdot COOH$$

while they digested only 40 grammes of crude protein. Therefore author has thought that the production of hippuric acid in animal body should be attributed in greater part, to the carbohydrates of food taken and glycocoll contained in hippuric acid should not only be produced from food glycocoll but also changed from other amino acid of food protein.

9. Calculation of apparent digestibilities of hay ashes showed that:

Chlorine	was digested	98 %
Potash	〃 〃	86 %
Sulfuric acid	〃 〃	66 %
while,		
Soda	was digested	only 20 %
Lime	〃 〃	〃 12 %
Magnesia	〃 〃	〃 15 %
Iron oxide	〃 〃	〃 18 %
Phosphoric acid	〃 〃	〃 15 %

10. Each sheep showed positive balance of every mineral matters except soda and magnesia, and one sheep showed negative balance of chlorine. The mixed hay used was contained too much of potash compared to soda and a little exess of lime compared to magnesia. It also cantained much of silicic acid. Therefore if sheep are to be fed on hay alone, it will be advisable to give additional amounts of salts containing small amount of magnesia (impure salts).

11. Finally, the author calculated the proportion of hay ashes distributed in urines, feces and balances. Potash was excreted 80–88% in urine and only 11–16% in feces, while soda was excreted only 25–29% in urine and 80% in feces. Lime was excreted almost completely in feces. Magnesia was also excreted largely in feces but it was excreted 18–28% in urine. Both phosphoric acid and iron oxide were excreted almost completely in feces. On the other hand, chlorine was excreted completely in urine. Sulfur was excreted equally in urine and in feces.

ON THE APPLICTION OF THE POLAROGRAPH TO THE ANALYSIS OF ABNORMAL MINERAL CONSTITUENTS.

By MASUZO SHIKATA, ISAMU TACHI and NOBUSHIGE HOZAKI

(*Agricultural Chemical Laboratory, Faculty of Agriculture, Kyoto Imperial University.*)

(Received Mar. 10th , 1927).

The applicability of the mercury dropping cathode and the Polarograph to the microanalysis of metallic ions has been suggested by Prof. Heyrovský and his colaborators. The present authors has tried to show to what extent this method can be applied to the biochemical problems.

Prof. Ikutaro Hirai has proved the menigitis-like disease of the infants, often seen in Japan, must be caused by the lead poisonning. This is shown by the presence of lead in brain, lung, heart, epiphragma, milt, gaster, intestine, liver, bone, hairs, urin and feces. However, the present authors have been told that no lead has been detected in 20 to 30ccm. of the cerebrospinal fluid of the patients.

The cerebrospinal fluids analysed in our experiments were 20 to 50ccm. taken from the patients, and have been forwarded by Prof. Hirai after he has evaporated and ignited the fluids under 500°C.

This ash has been extracted with 25ccm. of 1 n NaOH and electrolysed with the polarograph.

By the "polarogram" thus taken, the lead has been detected by the wave of the current voltage curve.

Among 9 samples, 0.006mg. to 0.015mg. Pb has been detected in 6 samples, and its trace in one sample, while no lead has been proved in two other sample.

As the second example, the Cu in the canned green peas has been determined. Cu is used as the colouring stuff to the green peas, fixing chlorophyll as copper phyllocyanate.

In this case 0.135mg. of Cu has been found in 2.319g. of green peas, i. e. 58.3mg. in Kg sample.

The limit of the concentration of Cu, which can be detected by the polarograph with the galvanometer of 10^{-8} amp. is 0.02mg. in 50ccm. i. e. 10^{-5} normal solution.

So it is quite easy to determine copper when we take 2g. of green peas.

This result has been compared by the ordinary electroanalysis by taking

40.3g. of green peas and it has been found that 1.7mg. copper in the sample, i. e. 37.13mg. per Kg sample.

This difference suggested us, that there must have been some loss of copper in the electrolysing solution. The residual solution after electrolysis, has been tested with the polarograph.

The polarogram showed that there was 0.7mg. Cu remaining in the solution, i. e. 15.29mg. per Kg sample.

Thus the sum of these two results is 52.42mg. So the electroanalysis, when corrected the loss of Cu in the solution after electrolysis is in fair accord with the polarographic measurements, i. e. discrepancy of about 10%.

From these two examples the applications of polarograph to the micro-analysis of abnormal inorganic constituents are shown to the satisfactory extent.

RESEARCH ON REDUCTION POTENTIALS OF ORGANIC COMPOUNDS. (Part II.)

REDUCTION POTENTIAL OF PYRIDINE.

By MASUZO SHIKATA and ISAMU TACHI.

(*Agricultural Chemical Laboratory, Faculty of Agriculture, Kyoto Imperial University.*)

(Received Mar. 10th., 1927.)

Summary.

(1) Reduction potential of pyridine has been measured with the dropping mercury cathode and the "Polarograph".

(2) Reduction potential of pyridine has been followed in acidic, neutral and alkaline solution.

(3) Reduction of pyridine proceeds in some way similar to the reversible reduction. However, reduction potentials, when compared with those, calculated by the Nernst formula, show much greater deviations than in the cases of reversible reductions such as isovaleraldehyde and nitrobenzene.

(4) Two waves of reduction have been observed in an acidic and in a neutral solution, the first wave is proved to be the reduction of pyridine ion, and the second to be that of undissociated molecule of pyridine.

Such a distinction could not have been established by the ordinary electrolytic measurement of oxydation-reduction system.

ON THE EGG-YOLK OIL.

By Kôzô Suzuki.

(*From the Chemical Laboratory of Imperial Zootechnical Experiment Station, Chiba.*)

(Received Mar. 21st., 1927.)

Having made a comparative study on the general properties and the kinds of fatty acids of the yolk of the eggs, which were producted by two different breeds, single comb White Leghorn and Chinese hens, the writer obtained the following results.

Egg-yolk oil has a special rank odour resembling that of cod liver oil and its colour is brownish yellow.

At the ordinal temperature, it is a liquid containing many fine needle formed crystallines, but it almost solidifies in winter.

General properties.

	White Leghorn	Chinese hens
Special Gravity (at 16°C.)	0.9767	0.9787
Refractive Index	1.4565	1.4690
Acid Value	8.82	10.43
Reichert–Meissl Value	0.88	0.88
Saponification Value	192.3	196.0
Iodine Value	70.7	70.1
Unsaponifiable Matter (%)	8.46	13.90

Properties of mixed fatty acids.

Solidifying Point	35.8°C.	34.0°C.
Melting Point	39°–42°C.	38°–39.5°C.
Neutralization Value	191.88	202.50
Mean Molecular Weight	292.37	277.04
Specific Gravity (at 15°C.)	0.9576	0.9581
Iodine Value	80.63	73.64
Amount of Saturated Fatty Acids (%)	30.41	32.15
Amount of Unsaturated fatty acids (%)	69.59	67.85

Properties of mixed saturated fatty acids.

Melting Point	54°–57°C.	51°–55°C.
Solidifying Point	53°C.	49°C.
Neutralization Value	211.6	219.7
Mean Molecular Weight	265.12	255.34

Properties of Mixed Unsaturated fatty acids.

Specific Gravity (at 15°C.)	0.9679	0.9665
Iodine Value	91.16	94.97
Neutralization Value	163.2	192.5
Mean Molecular Weight	290.37	291.43

About 2/3 of the total amount of the saturated fatty acids is isopalmitic acid (m. p. 57°C.) and 1/3 is palmitic acid (m. p. 62°C.).

Stearic acid is found to contain in the yolk oil of the eggs of White Leghorn but its amount is only 4 percent of saturated fatty acids.

Unsaturated fatty acids consist of the great amount of oleic acid and a very small quantity of linolic acid.

There exists also a very small amount of arachdonic acid in the yolk oil of Chinese eggs.

This difference regarding the constituents of the egg-yolk oil may be due to the kinds of feed, with which hens are fed, and not due to the difference of species.

ON THE DISTRIBUTION OF ACETYLMETHLCARBINOL AND 2, 3-BUTYLENE GLYCOL IN JAPANESE FERMENTATION PRODUCTS.

By Yukio Tomiyasu.

(*From the Agricultural Chemical Laboratory, Department of Agriculture, Kyushu Imperial University, Fukuoka.*)

(Received Apr. 10th., 1927.)

Since the production of acetylmethylcarbinol and 2, 3–butylene glycol by various microorganisms has often been reported by many investigators, the author supposed a wide distribution of these substances in fermentation products, and surveyed them by the modified method of Lemoigne. The results were summarized as follows :–

1. The occurrence of acetoin and 2, 3–butylene glycol was detected in various fermented vinegars. The acetoin-content of rice-vinegar was so larger than that of spirit-vinegar, that the sort of the vinegar could easily be differentiated by estimating the amount of acetoin. The spirit-vinegar, moreover, does not contain any 2, 3–butylene glycol, so that they could be distinguished clearly through its existence.

2. These substances were also found in saké. Their production seems to be mainly due to the action of saké-yeasts and probably not to the lactic acid bacteria. Attempting to distinguish the diseased saké or "Hiochi" from the sound by the estimation of the acetoin and 2, 3–butylene glycol-content,

the author found that their amount was generally richer in the diseased sakê than in the sound. The attempt to distinguish them, therefore, resulted in vain. This result shows that some of the "Hiochi" bacteria produces them.

3. The author detected the existence of them in soy and strawberry-wine, while 2, 3–butylene glycol was proved alone in beer, wine, and "Miso", which is a very common food made from soy beans, rice or barley, common salt, and water.

4. Acetoin combines with sulphite as well as aldehyde, (Neuberg ; Biochem. Zeitschr. **140**, 299, 1923), so that when the Ripper's method being applied for estimating aldehyde in the fermentation products which contain acetoin, it must be taken care upon this fact.

In conclusion the author wishes to express his thanks to Prof. Dr. M. Yukawa, Prof. Dr. Y. Okuda, and Ass. Prof. I. Yamasaki for their kind direction and advice throughout the investigation.

ON THE WEATHERING OF THE GRANITE-ROCKS OF TWO DISTRICTS IN JAPAN.

By SHIGERU OSUGI and TAKEO TANAKA.

(*Dept. of Agr. Kyoto Imp. Univ.*)

(Received Apr. 11th., 1927.)

1. The weathering of the granite-rocks collected in Kyoto and Kagawa Prefectures in Japan, was investigated.

The climatic conditions of the districts noted above are as follows :

District.	Annual temperature (°C)			Annual rain-fall (m.m.)
	Max.	Min.	Ave.	
Shirakawa, Kyoto, Japan.	19.94	8.45	13.76	1587 58
Kida-mura, Kagawa, Shikoku, Japan.	19.50	10.50	16.60	1185.60

2. The composition of the fresh rock and the weathered products of various stages was determined.

The calculation on the loss and the gain of each constituent during the weathering process, was made after Merrill's method.

The results thus obtained, were compared with those reported by Merrill, as shown in the following table ;

District.		% loss of each constituent.														% total loss to entire rock	
		SiO_2		Al_2O_3		Fe_2O_3		CaO		MgO		Na_2O		K_2O			
			b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Shirakawa, Kyoto, Japan.	B	5.68	23.29	0	13.29	15.35 gain	0	39.13	47.23	28.32	46.53	47.28	54.29	41.61	49.37	15.37	26.50
	C	5.37	17.76	0	7.38	7.38 gain	0	41.58	45.16	56.55	59.74	71.79	73.86	68.61	70.91	18.41	23.74
	D	1.50	16.87	0	10.03	11.66 gain	0	53.66	58.32	54.49	59.06	70.18	73.17	60.25	64.24	16.06	23.51
Kida-mura, Kagawa, Japan.	B	25.20	2.82	0	29.85 gain	25.30	0	35.00	15.60	27.20	5.42	68.40	58.97	39.80	21.78	25.19	0.78
	C	7.81	23.27	0	15.86	18.80 gain	0	39.10	48.79	27.70	39.15	64.50	70.14	26.40	38.07	10.56	24.02
	D	5.23	21.59	0	17.22	20.79	0	55.30	63.01	35.90	46.94	78.10	81.89	18.10	32.16	8.00	23.57
District of Columbia, U.S.A.(1)		—	14.89	—	3.23	—	0	—	25.21	—	14.90	—	28.62	—	31.98	—	13.78
Virginia U.S.A.(2)		—	52.45	—	14.39	—	0	—	100	—	74.70	—	45.03	—	83.52	—	44.67
Georgia, U.S.A.(3)		—	77.20	—	43.82	—	0	—	98.78	—	87.94	—	92.16	—	91.75	—	71.84

Remarks:

a.·········Calculated on assumption that alumina has not been lost at all.

b.········· 〃 〃 〃 ferric oxide 〃 〃

B.·········First stage. (weathered rock block) C.·······Second stage. D. · ···Residual soil.

(1)·······Example of the disintegration of granite. (Residual soil only)

(2)·······Example of the chemical weathering of granite. (Residnal soil only)

From the above table, it may be assumed that the predominating factor of the changes taking place in the rocks under investigation, is rather physical than chemical nature although the latter is not ignored.

3. The change of the mineral composition was then investigated and the results obtained are noted in the following table.

The table shows that even feldspar and biotite which are supposed to be rather easily decomposed chemically, were not changed to any extent.

Only in case of the biotite, a distinct loss is recognized although the loss is not so marked as to be expected from the chemical weathering.

	Kyoto, Japan(1)			Kagawa, Japan(1)		
	Quartz.	Feldspar.	Biotite.	Quartz.	Feldspar.	Biotite.
A	1	0.63	0.42	1	0.49	0.31
B	1	0.61	0.27	1	0.64	0.36
C	1	0.79	0.35	1	0.40	0.34
D	1	0.97	0.26	1	0.59	0.09

[(1) In this table, only the ratio of mineral is shown.]
A············fresh rock. B············first weathering product.
C············second weathering product. D············residual soil.

4. The mechanical composition of the weathered products was tested, and obtained the following results which indicate that the products are mainly consisted of coarser particles such as the gravels and the coarse sands, and only a very small quantity of clay is found in the products.

	Kyoto, Japan.		Kagawa, Japan.		District of Columbia. U.S.A.	
	gravel and coarse sand.	fine sand and silt.	gravel and coarse sand.	fine sand and silt.	gravel and coarse sand.	fine(1) sand and silt.
B(2)	85.00%	14.00%	95.43%	4.57%	—	—
C	83.05	14.57	84.26	16.23	—	—
D	61.70	30.39	82.57	17.86	78.00	22.00

(1) Example of the disintegration of granite.
(2) B is powdered mechanically.

The above results show again that the granites named above were changed mainly physically and not much chemical change took place.

5. From the results obtained in three foregoing investigations, it may be concluded that the predominating change taken place in the weathering process of two granites tested, is chiefly physical although the chemical change is considered at the same time.

(From the Laboratory of Division of Soil, Kyoto Imperial Univ. Kyoto, Japan.)

STUDIES ON THE ACIDS FORMED BY RHIZOPUS SPECIES. PART V.

By TEIZO TAKAHASHI and KINICHIRO SAKAGUCHI.

(Received Apr. 18th., 1927.)

In the first report,[1] authors have stated the occurrence of malic acid in organic acids formed by Rhizopus species, by the tests rather specific to the acid, and later it was isolated and accurately identified by Dakin's method.[2]

Since then, the formation of malic acid by this fungus was affirmed not only from glucose, saccharose, starch or the like, as a cleavage product, but also from some simple compound such as fumaric acid as a synthetic product, in very similar way as formation of succinic acid from acetic acid thus.

Rhiz. G. 34, Fumaric acid. 2g. *l*-malic acid. 0.101g.
and 0.132g. as cinchonin malate.

The formation of fumaric–, lactic acid and some volatile acid and ethyl alcohol by this fungus was proved to be the normal products from glucose or other carbohydrates but these also comes to met with when malic acid is given as a carbon source, as is shown in the following table.

Fungus species.	Temp.	Duration of Calture.	Fungus growth(g.) dry m.	Volatile acid. $N/_{10}$ NaOH. to 10c c. medium.	Fumaric acid(g.).	Lactic acid.	Ethyl alcohol W. %	Malic acid. remained. (g.)
Rhiz. G. 36.	21–32°C.	72	0.354	0.5	0.125	+	0.16	+
Rhiz G. 36.	25–30°C.	45	0.310	0.6	0.24	0 09g. as Ba. salt.	+	0.671
Rhiz. G. 34.	21–32°C.	72	0.725	0.5	0.06	±	0.11	+
Rhiz. G. 34.	25–30°C.	45	0.281	1.0	0.43	±	+	0.503

---

(1) Journ. of The Agric. Chem. Society of Japan. Vol. I. No. 5.
(2) Journ. of The Agric. Chem. Society of Japan. Vol. II. No. 5.

Hence, the mechanism of the formation of fumaric-, lactic acid and ethyl alcohol from malic acid may be represented most probably in accordance with following equations :–

a) $$2\begin{array}{l} CH \cdot OH \cdot COOH \\ | \\ CH_2 \cdot COOH \end{array} = 2\begin{array}{l} CH_2 \cdot COOH \\ | \\ CH_2 \cdot COOH \end{array} + O_2$$

b) $$\begin{array}{l} CH_2 \cdot COOH \\ | \\ CH_2 \cdot COOH \end{array} = \begin{array}{l} CH \cdot COOH \\ \| \\ CH \cdot COOH \end{array} + H_2$$

c) $$\begin{array}{l} CH \cdot COOH \\ \| \\ CH \cdot COOH \end{array} + \begin{array}{c} H \\ | \\ OH \end{array} = \begin{array}{l} CH_3 \\ | \\ CH \cdot OH \\ | \\ COOH \end{array} + CO_2$$

d) $$\begin{array}{l} CH_3 \\ | \\ CH \cdot OH \\ | \\ COOH \end{array} = \begin{array}{l} CH_3 \\ | \\ CO \\ | \\ COOH \end{array} + H_2$$

e) $$\begin{array}{l} CH_3 \\ | \\ CO \\ | \\ COOH \end{array} = CH_3 \cdot COH + CO_2$$

f) $$CH_3 \cdot COH + H_2 = CH_3 \cdot CH_2 \cdot OH$$

The first equation viz., the occurrence of succinic acid in the products was not affirmed by this chance, but it is most probable to assume the acid as an intermediate product, as we have already stated, when gluconic acid is given as only carbon source of the culture medium.

The quantity of ethyl alcohol found was too small to be mentioned. This is nothing but that we have limitted or rather minimamized the quantity of nitrogen source of nutrient viz., the most unfabourable condition for the nomal evolution of alcohol.

The synthetic formation of malic acid from fumaric acid is most simple and the nature of the change which ensue may not be other than the following representation :

$$\begin{array}{l} CH \cdot COOH \\ \| \\ CH \cdot COOH \end{array} + \begin{array}{c} OH \\ | \\ H \end{array} = \begin{array}{l} CH \cdot OH \cdot COOH \\ | \\ CH_2 \cdot COOH \end{array}$$

From these facts, it seems to us that fungus aught just to get nothing else but the energy for its growth, irrespective whether synthesis or decomposition of the substances is going on in the medium.

Experimental.

I) The formation of fumaric acid and other substances from malic acid.

The culture medium constituted of; distilled water 150c.c., malic acid*

* Pure material from Märck's brand.

3g., 10% NH_4OH. 15c.c., $CaCO_3$ 4g., K_2HPO_4 and KH_2PO_4 each 0.0225g., $CaCl_2$ and $MgSO_4$ each 0.015g., NaCl and Fe_2Cl_6 each trace.

Volatile acid was determined with the distillate of steam destillation of 10c.c. of the culture medium.

Fumaric acid was isolated from 150c.c. of the culture medium by the extraction by ether, as already stated several times, after the removal of alcohol by distillation, as shown below :–

	From Rhiz. G. 36.	From Rhiz. G. 34.	Calcul.
Melting point.	281–284°C.	280–284°C.	—
Number of titration (0.1g.)	17.2c.c. $N/_{10}$NaOH	17.2c.c. $N/_{10}$NaOH	17.24c.c.
Silver salt.	0.0817g.	0.0691g.	—
AgCl.	0.0709g.	0.0059g.	—
Ag.	65.30%	65.25%	65.44%

Malic acid, unassimilated, was isolated from soluble part of ether extract stated as above, by the nutralizing with baryta water followed by the addition of 80% of alcohol, which dissolving lactate and remaining behind malate as a precipitate.

Lactic acid. Qualitative tests were made about barium salt, which comes in solution in 80% alcohol as mentioned above, after Hapkin's Denige's[(3)] and authors[(4)] reactions.

Aldehyde was detected in the neutral distillate by Schiff's reaction and Na–nitroprussid and aqueous alkali.

II) The formation of *l*–malic acid from fumaric acid.

The culture medium used was :– Fumaric acid 2g., $(NH_4)_2SO_4$ 0.5g., $CaCO_3$ 3g., K_2HPO_4 and KH_2PO_4 each 0.015g., $CaCl_2$ and $MgSO_4$ each 0.01g., NaCl and Fe_2Cl_6 each trace, distilled water 100c.c.

The duration of the culture was 42 days at room temperature.

l–Malic acid :– To isolate the acid the culture medium was extracted, as stated several times, with ether and from this extract the soluble part in water was separated from unaltered fumaric acid, and from aqueous solution hygroscopic crystalls appeared after recrystallization from water. This crystalls gave a heavy white precipitate by Denige's reagent and reduced palladium chloride. Its melting point was 99–100°C, which is accurately

(3) Guajacol and H_2SO_4 (Denige'. Zeit. f. Anal. Chem. 50. 1911, 189).

(4) β–Naphtol and H_2SO_4 (Journ. of Agric. Chem. Soc. Japan. Vol. I. No. 14)

(5) Dakin. Journ. Biol. Chem. Vol. LIX. No. 1. p. 7. 1924.

(6) Pasteur. A., 82, 331, He gave 100°C.

same as that of given by Dakin or Pasteur. Cinchonin-malate prepared after Dakin's method melted at 198–199°C.

The enhanced rotatory power was determined by adding uranium acetate with the results as shown below :–

Substance	0.335%
Temperature	23°C
$\alpha = -1.47$	$l = 1$cm.

$$\therefore \quad [\alpha]_D^{23} = \frac{-1.47\times 100}{0.335\times 1} = -438.8°$$

For the comparision, Yoder's[(7)] and Dakin's[(8)] data should be quoted below :–

After Yoder, $C = 1\%$
$[\alpha]_D^{20} = -501°$
After Dakin, $C = 0.5673, \quad l = 2.2$
$\alpha = -6.02°$
$[\alpha]_D^{18} = -482°$

(7) Yoder: Zeit, f. Nahr. u. Genuss, 22. (1911), 329.
(8) Dakin: ibid.

HYDROGENATION OF STEROL-FREE UNSAPONIFIABLE MATTERS OF COD-LIVER OIL. I.

By Ziro Nakamiya and Kozo Kawakami.

(*From the Institute of Physical and Chemical Researches, Tokio.*)

(Received May 28th., 1927.)

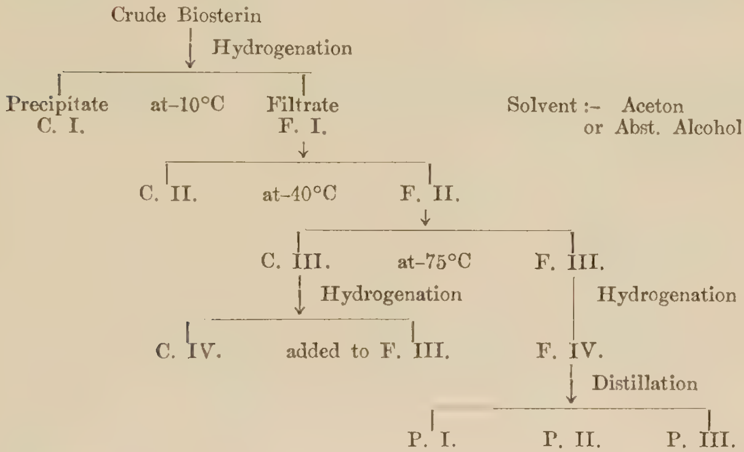
The authors had reported with Dr. Katsumi Takahashi on the physical and chemical properties of Biosterin (a name given to Fat-soluble A) in the Bulletin and the Scientific Papers of their Institute.* A further study has been continued by the present authors on the hydrogenated products of crude Biosterin. They have investigated independently and compared their results in order to ascert ain their experiments.

The conclusion was as follows :–

* K. Takahashi, Z. Nakamiya, K. Kawakami, & T. Kitasato :–
Bulletin of the Inst. of Phys. & Chem. Researches. Vol. 3, (1924) No. 6.
K. Takahashi, Z. Nakamiya, K. Kawakami, & T. Kitasato :–
Scientific Papers of the Inst. No. 32 (1925).

1. On hydrogenation of crude Biosterin, some of it changed into a crystalline mass, from which the following substances were separated.

Method of hydrogenation :–	By Fokin and Willstäter.	
Catalyser :–	Palladium black, by Tausz and Putnoky's method.	
	Nakamiya	Kawakami
Solvent :–	Glacial acetic acid	Acetic ether
Temperature of reaction	60°C	Room temperature



The following two were found in both (N. & K.'s) samples.

Nonacosane $C_{29}H_{60}$

m. p. a) 61.5–62°C b) 62–63°C by N. c) 62°C by K.
Analysis (Micro.) :–

			C%	H%
Sample a)				
(1)	0.003026g.	Found	85.29	15.12
(2)	0.003220g.		85.20	14.78
		Average	85.25	14.95
Sample b)				
(1)	0.002910g.	Found	85.22	15.05
(2)	0.003240g.		85.27	15.15
		Average	85.25	15.10
$C_{29}H_{60}$ (m. p. 62–63°C)		Calc.	85.30	14.70

Molecular weight (By Rast's Camphor Method)

Found	428.4	382.2
Calculated	408.0	

Batyl alcohol $C_{21}H_{44}O_3$

m. p. a) 58°C b) 56–57°C by N. c) 62°C by K.
Analysis (Submicro.) :–

			C%	H%	C%
	Sample a)				
(1)	0.0434g.	Found	72.77	12.06	15.17
	Sample b)	(Micro.)			
(2)	0.003160g.		73.19	13.19	13.80
(3)	0.003170g.		72.91	12.51	14.58
	Sample c)				
(1)	0.1094g.		73.24	12.88	13.88
(2)	0.1165g.		73.69	12.95	13.36
$C_{21}H_{44}O_3$ (m. p. 70–71°C)					
	Calculated		73.18	12.88	13.94

Melting point of the author's samples is yet lower than that of batyl alcohol by Dr. Toyama. The following substances were found in N's samples.

Octadecyl alcohol palmitic acid ester

m. p. 58–59°C
Analysis (micro) :–

	Sample		C%	H%	O%
(1)	0.002925g.	Found	80.14	13.20	6.66
(2)	0,002970g.		80.21	13.41	0.38
		Average	80.18	13.31	6.51

	m. p.	mol. w.	C%	H%	O%
Octadecyl alcohol	59°	270.00	79.91	14.17	5.92
〃 palmitic acid ester	59°	508.52	80.3	13.4	6.3

Molecular weight (By Rast's method)
527.9 518.8

Myricyl alcohol (Melissyl alcohol) $C_{30}H_{61}OH$

m. p. 84°C
Analysis (Micro.) :–

	Sample		C%	H%	O%
(1)	0.003080g.	Found	81.91	14.01	4.08
(2)	0.003070g.		82.26	14.16	3.58
		Average	82.09	14.09	3.82
$C_{30}H_{61}OH$ (m. p. 85°, or 88°)					
($C_{31}H_{63}OH$)		Calc.	82.21	14.25	3.54

Molecular weight (By Rast's method)

Found	449.4
Calc.	452.51

Unknown saturated alcohol

m. p. 89–91°C
Analysis (Micro.) :–

Sample		C%	H%	C%
(1) 0.003060g.	Found	79.95	13.90	6.15
(2) 0.003145g.		80.08	13.43	6.49
	Average	80.02	13.67	6.32

Molecular weight (By Rast's method)

Found 454.3 511.1

$C_{34}H_{68}O_2$ (=508) ? C 80.3%, H 13.4%, C 6.3%.

All of these were not always found in each sample used, even after repeated experiments. These results were obtained from many samples during 1925–1927.

Before treatment the original unsaturated compounds in the crude Biosterin may perhaps exist in liquid form, but the authors cannot yet succeed to separate these compounds from Biosterin, for their solubility and boiling point hardly can be distinguished from those of Biosterin.

2. The most part (90–95%) of the hydrogenated product of crude Biosterin was in liquid state, and the authors could separate it by distillation into two mainly different portions, though the separation may not be yet completely perfect. The one portion: (6), (7), (8), (10), contains more than 80% carbon and 12–13% hydrogen; another portion (9) about 77% carbon, and 12% hydrogen. The latter distils out at a higher temperature than that of the former.

By Nakamiya :–

	Sample.	C %	H%	O %	Mol. W.	Iodine Value Wijs'	Ref. Index Abbe's
1	Crude Biosterin	80.80	10.62	8.58	—	—	—
2	C. III. Hyd. ppt.–75	81.48	12.27	6.25	—	146.6	—
3	C. IV. Hyd. & Hyd. ppt.–75	81.27	13.05	5.68	—	21.01	1.4705
4	F. III. Hyd. Non ppt.	79.88	11.79	8.33	—	156.5	—
5	F. IV. Hyd & Hyd.	79.36	12.65	7.99	—	30.96	—
6	Distillate I of F. IV.	80.27	13.03	6.70	—	13.62	1.460(40°)
7	Dist. II. of F. IV.	80.28	13.13	6.59	—	—	1.465(40°)
8	X. 80–175°(2mm.)	80.09	12.89	7.02	243.7	33.98	1.465(20°)
9	Y. 175–195°(2mm.)	77.19	12.29	10.62	296.4	31.90	1.469(20°)
10	L. 70–175°(2mm.)	79.99	12.79	7.32	240.4	47.80	1.4680(20°)

By Kawakami :–

	Fraction & B. P.	Yield	C %	H%	O %	Mol. W.	Iodine Value Wijs'	Form and Color
1	130–205(15mm.)	1g.	80.77	11.86	7.37	—	104.5(4h)	Light yellowish Liquid
2	205–210	4	81.31	12.40	6.29	277	95.9	Light yellowish oil
3	225–230	6	81.08	12.35	6.57	—	94.9	〃 〃
4	230–270	3	77.22	12.55	10.23	—	93.8	Browhish yellow oil
5	Residue	9	77.75	11.46	10.79	—	104.0	Dark brown syrup

3. During the purification of crude Biosterin, the authors obtained certain substances precipitable in aceton at –75°C. The most part of these were found as the mixture of cholesterol and a little Biosterin. Besides these, N. separated an unsaturated alcohol with m. p. 72–73°C. This unsaturated alcohol after hydrogenation changed into a saturated alcohol with m. p. 89–91°C, which already described above. K. obtained such a substance something like chymilalcohol.

4. N. confirmed, by feeding experiments, the decreasing of activity of vitamin A by hydrogenation, and also found that the activity of Biosterin may be lost if it is saturated with hydrogen atoms completely.

EXPERIMENTAL STUDIES UPON THE ALKALIMETRIC ESTIMATION OF AMINO ACIDS AND PEPTIDES BY THE METHOD OF WILLSTÄTTER AND WALDSCHMIDT-LEITZ.

By Etsuo Takamiya.

(*Biochemical Laboratory, Department of Agriculture, Kyushu Imperial University, Fukuoka.*)

(Received Apr., 22nd., 1927.)

It was found by D. Vorländer[1] that glycocoll in alcoholic solution behaves as acid against phenolpthalein. Applying this fact Willstätter and Waldschmidt–Leitz[2] established a new method for the estimation of amino acids and peptides at a definite alcoholic concentration by alkalimetry.

Sörensen's Formol titration method has hitherto been adapted for estimating amino acids and peptides, although it requires considerable trouble. They, however, are readily estimated by the use of the W. and W. method.

I have studied the effects of temperature, dilution, indicator, acid and alkali and neutral salts upon this method, and also the adaptation of this method in the presence of acid and alkali and neutral salts, with following results.

1) There is no influence of temperature upon this method.

2) In the dilution experiments, one titration value was compared with another; the one was obtained by the immediate titration of amino acid

(1) Ann. d. Chem., **341**, 1, (1905)

(2) Ber. d. Deutsch. Chem. Ges., **54**, 2988, (1921)

(3) Ber. d. Deutsch. Chem. Ges., **52**, 309, (1919)

solution of definite alcoholic concentration which had been allowed to stand for a difinite time, and the other by the titration of the amino acid alcoholic solution after dilution with water ; no recognizable discrepancy was practically observed between them. From these experimental data it seems that there is no need especially to use an alkali-alcoholic solution for the titration.

3) From the fact obtained that an alcoholic solution of amino acids does not behave as acid against both rosolic acid and p–nitrophenol which have been adapted as indicators in place of phenolphthalein, it was ascertained, unlike the assumption of Vorländer[(3)] or Willstätter, that this phenomenon is entirely due to the effect of amino acid or peptide-alcoholic solution upon the indicator, phenolphthalein.

4) It was found that the titration value for amino acids is depressed in the presence of acid or alkali :– (a) the higher the concentration of acid or alkali, the greater is the degree of depression. (b) The degree slightly increases in the following order of NaOH, CH_3COOH and HCl. (c) Alanine is depressed to a slightly greater degree than glycocoll. (d) The lower the alcoholic concentration of the solution, the greater is the degree of depression.

5) This method can not be immediately adapted in the presence of acid and alkali. In such a case my experimental results confirm that amino acids and peptides are exactly by this method if the following procedures carried out : First, add 0.1c.c. phenolphthalein (1 %) to the sample solution to be estimated, neutralise until a distinctly pink color is obtained, and then make to a difinite alcoholic percentage ; add again 0.9c.c. phenolphthalein and titrate it with a standard alkali solution until a distinctly pink color is obtained. The amount of alkali thus obtained corresponds to the quantity of amino acids and peptides.

6) It was found that Mn-chloride, Mn-sulphate, Mg-chloride, Mg-sulphate, and Mg-nitrate behave as acid against phenolphthalein in their alcoholic solution. Since the presence of these neutral salts misleads this method, it is necessary to remove them as their hydroxide by alkali before applying it.

ON THE MANGANESE CONTAINED IN THE MULBERRY LEAF.

By Shozo Bito.

(Received May 5th., 1927)

The manganese has in recent times been recognized as one of the essen-

tial elements in the growth of the plant.

The author has investigated the manganese contained in the leaf and flower of the mulberry tree.

The summary of the experimental results may be given as follows.

1. The mulberry leaf contains a certain amount of manganese, this suggests that possibly this metal may play a role in metabolism.

2. The manganese in the mulberry leaf increases according to the growth of the leaf.

3. The manganese contained in the mesophyll is greater than that in the vein and petiole.

4. The flower of the mulberry contains about the same amount of manganese as that of the leaf.

5. The quantity of manganese contained in the leaf varies according to the variety of the mulberry, in spite of its being cultivated in the same environment.

6. It is probable that the higher amount of the manganese has some connection with the discolouration of the leaf in the late autumn.

STUDIES ON ENZYME ACTION III.

ON THE SELECTIVE ADSORPTIONS OF UREASE AND PROTEINS IN A MIXED SOLUTION. (1)

By Matsunosuke Kitagawa.

(Received May 4 th, 1927)

For the purpose of applying the adsorption method to the purification of enzyme, it seems, as a rule, to be of the first importance to study the essential characters of the selective adsorptions of enzyme and proteins which exist as a mixture in the crude enzyme solution. Consequently I compared their adsorptive natures in a enzyme solution, which was obtained by the preliminay purification of the original urease extract from soy beans or jack beans, and examined the conditions favourable to their selective adsorptions. Finally I tried to establish a correlation between urease and some bean proteins, which has been previously discussed by J, B. Sumner, and came to the following conclusions.

A. On the preliminary purification.

1) Jack beans contain on the average fifteen times as much urease as soy beans, and the extraction of the urease from the materials with five times

their weight of water gave the best yield.

2) The protein-precipitant, such as lead acetate, removed over 85 per cent of protein in the original extract, but also at the same time about 65 per cent of the urease, so that the precipitant can not be used for the isolation.

3) From 27 to 47 per cent of the urease, which was adsorbed by the proteins precipitated with alcohol, was set free from the precipite by shaking with water, and about 70 per cent of it with baryta. In every case more or less protein was also dissolved.

4) As a result of the experiments, it was found that dialysis of the dilute original extracts in a collodion sac is the best method for the preliminary purification of the urease. By this method a solution containing about 80 per cent of urease and 12 per cent of proteins of the original extracts was obtained, the loss of urease being about 20 per cent, of which 5 per cent was by dialysis and 15 per cent by adsorption.

B. Experiments on Adsorption

1) The adsorption-value of the urease and proteins, namely the quantity of the substances adsorbed by 0.1g. of Al_2O_3, was studied with the above mentioned urease solution. In the case of the protein the adsorption-curve was approximately equal to Freundlich's adsorptionisotherm, but in the case of the urease, the larger the concentration of the extract, the more rapid was the decrease of the adsorption-value, as shown in the following table. The reason for this depends upon the fact that in a concentrated extract protein has stronger adsorptive power than urease, consequently a much larger surface of the adsorbent is occupied by the proteins, and the urease is expelled.

	enzyme solution				adsorption value		adsorption %	
	urease conc.		protein conc.					
Al_2O_3 %	before adsorp.	after adsorp.	before adsorp.	after apsorp.	urease	protein	urease	protein
1.31	269	10	248	37	200	16.2	96	85
0.97	〃	95	〃	51	179	20.2	64	79
0.66	〃	221	〃	99	74	22.9	17	60
0.33	〃	269	〃	165	0	25.3	0	33

(Remarks: Each unit used for urease and protein is different)

From the experimental results we see that the quantity of adsorbent is considered to be the neccessary factor controlling the selective adsorption, that is, by the use of a large amount of adsorbent comparatively more urease is adsorbed; and on the contrary, by the use of a small amount of it comparatively more protein is adsorbed.

2) When the solution was diluted, no effect was observed on the adsorption of the protein, but that of urease was markedly increased.

4) As the urease and proteins, which exist in the purified enzyme

solution obtained by means of dialysis, gave very different adsorption-curves and showed a different manner of adsorption on dilution, it is concluded that they are not identical but only a mixture.

STUDIES ON ENZYME ACTION IV.

ON THE SELECTIVE ADSORPTIONS OF UREASE AND PROTEINS IN A MIXED SOLUTION. (2)

By MATSUNOSUKE KITAGAWA

(Received May 4th., 1927)

As a biological extract such as an enzyme solution contains always several kinds of salt in different quantities, the influence of salts upon the selective adsorption of urease and proteins by aluminium hydroxide was examined in jack bean-urease solution, with the following results.

1) Inorganic salts generally make a salt-like adsorption with aluminium hydroxide at the solid surface of the adsorbent, but in the presence of urease and proteins a part of the salts is replaced by them, therefore the mechanism of the adsorption of the urease and proteins by aluminium hydroxide should be ascribed to chemical affinity, not to merely mechanical adhesion.

2) The salts are divided into two groups from the point of view of adsorption. The one like phosphates occupies the active surface of the adsorbent and diminishes the adsorption of the protein and urease, especially of the latter. The other like NaCl or CH_3COONa does not occupy the surface but merely changes the nature of the medium, and exerts some influence upon the adsorption, namely in the presence of certain phosphates it increases the adsorption of urease, but in their absence it decreases the adsorption.

The adsorption of proteins and urease is most favorable in the solution containing no salts.

Thus the selective adsorptions of the urease and proteins are influenced quantitatively by the nature and quantity of salts present. Consequently, in the presence of a moderate quantity of the adsorbent, and in accomparatively pure solution obtained by complete dialysis the urease is more easily adsorbed, but on the contrary, in a less pure solution obtained by insufficient dialysis, the proteins are more easily adsorbed. In the latter case, however, if NaCl or CH_3COONa is added to the solution, the degree of selective adsorption is reduced and the adsorption of urease is promoted.

3) The adsorptions of these substances were completed instanteneously

in a pure solution, but, in the presence of some salt, required some short intervals of time for attaining equilibrium.

4) The influence of hydrogen ion concentration upon the adsorption was remarkable, especially in the case of urease, in the solution containing some salts, namely in an acid range such as pH 5, the adsorption was very much accelerated, but in an alkaline range such as pH 7.5 it was largely retarded. In this case, the less the concentration of the salts, the less was the influence.

Urease is an amphoteric substance, as it was readily adsorbed by both kaolin and aluminium hydroxide.

5) The urease and proteins were not set free from their adsorption-compounds either by shaking with water or with glycocoll, acetate or NaCl, but were set free only by the addition of phosphate. In this case the proteins were more easily liberated than the urease. The liberation or elution was very insignificant especially in the case of urease at pH 4.5, but gradually increased with increasing alkalinity up to pH 7.5.

6) I have repeated several times Sumners' experiments on the crystallization of urease from the aceton extract, but unfortunately failed to justify his findings. On the other hand, my urease preparation obtained by his method was shown to be less active than that obtained by the method of dialysis. Consequently, I used the latter perparation for the examination of adsorptive factors.

As a result the experiments in regard to the adsorption-curves, the salt-influences and other factors, we came to the conclusion that urease and protein are not identical but exist as a mixture even in the purest preparation examined.

ON THE DEGREE OF SATURATION AND THE ADSORBED BASES OF JAPANESE SOILS.

Shigeru Osugi and Yoshio Sano.

(Received May 17 th., 1927)

1) The dgree of saturation of Japanese soils was investigated by Hissink's method and it was found that the degree of saturation thus obtained was fairly in accordance with the reaction of the soils tested as shown in the following table.

soil.	(Degree of saturation)	Ph.
1	77.1	6.76
2	19.4	4.35

3	28.9	5.95
4	73.3	6.81
5	31.4	5.38
6	33.2	4.35
7	49.8	6.46
8	17.4	5.98
9	13.0	5.29
10	24.1	6.34
11	45.3	6.56
12	60.6	6.99
13	61.4	7.12

It was found that the soils were saturated when the surrounding liquid reacts distinctly alkaline as PH 8.6–11 as in the following table.

Soil.	1	2	3	4	5	6	7	8	9	10	11	12	13
PH	9.15	9.25	9.15	11.67	9.60	8.95	10.20	10.30	9.10	8.60	9.05	8.62	9.55

2) The adsorbed bases of the soils were tested by Hissink's method and were compared with those of Dutch soils reported by him as shown in the following table which shows that in the soils tested, the amount of adsorbed calcium is smaller and those of Mg & Na are greater than those in Dutch soils.

% composition of adsorbed bases (in mg. equivalent)

	Ca	Mg	K	Na
Average of all soils tested	61.20	26.04	2.71	10.69
Average of the soils whose PH are greater than 6.	76.47	4.44	1.62	7.05
Average of the soils whose PH are smaller than 6.	43.40	39.53	3.99	14.94
Average of Dutch soils.	76-79	13	2–3	6–8

In the case of the alkali–soils of Formosa–island, the composition of the adsorbed bases lies between those of the alkali–soil and the flooded soil reported by Page & Williams as follows;

% composition of adsorbed bases (in mg. equivalent)

		Ca	Mg	K	Na
Alkali–soil of	A	14.31	11.21	14.37	60.11
Formosa–island	B	39.60	33.61	3.98	22.81

Result reported by Page & Williams.

	Ca	Mg+K+Na
Normal Soil	90	10
Flooded soil	48	52
Alkali-soil	0	100

3) The correlation between the composition of the adsorbed bases and the physical properties of the soils was investigated and it was not found any distinct relation and this was ascribed to the rather similar nature of the soils tested and was hoped to test this point further. The alkali-soils in Formosa island contain a considerable amount of Na and they became very plastic when the soluble salts were washed.

ON THE MECHANISM OF THE FRUIT-ESTER-FORMATION BY WILLIA ANOMALA SP.

By Masakazu Yamada.

(Received Feb. 26 th., 1927)

It is a well-known character of Willa, Mycoderma and other few microbes that they produce fruit ester like ethylacetate in the various culture-media. T. Takahashi and H. Sato ascertained the fact with Willia anomala or aging yeast of saké, which was isolated from a deposit formed on the bottom of the vat during the storage period of saké, that ester flavour was formed from fermentable sugars, alcohl and ammonium-acetate originally existed in nutrient as a carbon source but from glycerine.(1) Lately U. Weber confirmed qualitatively with six kinds of organisms (Willia, Oidium, Pichia and Sachsia) that the flavour was produced in the culture media containing glucose, fructose, saccharose, maltose and the wort but not in the cases of lactose, dextrin, mannit and glycerine.

Further he showed that in the media of alcohol (methyl, propyl and butyl) preveously added to glucose yeast water the flavour of their acetic esters was felt and that in the media simply cotaining alcohol (propyl, butyl, isoamyl) their esters were formed but in the mixture of alcohols and organic acids their mutual esters were not formed. In similar experiments with the mixture of sugar and amino acid (glycine, alanine, leucine and tyrosine) only leucine gave the characteristic flavour of amylester(2). In saké brewing, willia sp. was said to be a kind of aging yeast but the flavour of ethyl ester produced by it was rather disliked. So it is interesting from the biochemical stand point and important for the practice to make clear the mechanism of ester formation by the organism.

The author found that willia was the most eminent species for aldehyde-production and that when aldehyde was held by means of the fixative, which was originally added to the medium containing either sugar or alcohol as a simple carbon source, no ester flavours were often recognized. Further experiments were made in the same direction and now it became obvious that in the ester formation the acid component should have prassed the aldehyde stage whether it came from sugar or alcohol. Thus, ethylalcohol and acetic acid from the fermentation of sugar and also from the secondary oxidation of alcohol may combine mutually forming ethyl acetate.

---

(1) J. Coll. Agri. Tokyo. **1**, 227—68, 1911.

(2) Bioch. Z. **129**, 208—16, 1922.

sugar→acetaldehyd→ethyl alcohol→acetaldehyde→ethyl alcohol
↘acetic acid ↘acetic acid
↘ Ethyl acetate ↙

Whether the acid stage ought to be passed or not, is not yet accurately known because the flxative action on $CaCO_3$ is imperfect.

The fact is applied for alcohols which are easily oxdized into corresponding aldehydes by yeasts. Propyl propinate was formed from propyl alcohol and butyl butyrate from butyl alcohol. Various esters may be formed from the mixture of alcohols, some of which are produced in the amino-acid fermentation.

Miximum yield of ester (ethyl acetate) was obtained after 8 days in the culture containing 20–25% of sugar and also after 20 days in 4% of alcohol solution. Above 12% of alcohol there is not perceived ester flavour and so no anxiety necessiates for saké which contains at least 15% of alcohol. In author's experiments any production of aldehyde or ester flavour did not result in the medium containing ammonium-acetate as both corbon and nitrogen source by willia.

Experimental

Microbes used is Willia anomala var. saké I, Takahashi in all cases.

1. Optimum condition for ester formation.

A. Age of culture. Medium. koji extract (15°B) 100c.c. Grams of total ester produced per 100c.c. of culture is as follows:–

days	original koji ex.	2	5	8	12	30
ester (g)	0.2182	0.3698	0.6242	0.6424	0.5584	0.3887

B. Optimum concentration of both sugar and alcohol.

Medium : a. Hayduck's solution.

b. Modified Hayduck's solution (alc. instead of sugar)

a		b	
Concentration of sugar %	total ester per 100c.c. g.	Concentration of alcohol %	total ester per 100c.c. g.
1	0.0968	1	0.0915
2	0.1302	2	0.1619
5	0.1848	4	0.3450
10	0.4206	6	0.2517
15	0.4664	8	0.3168
20	0.5245	10	0.1461
25	0.5086	12	0.0141
30	0.4136	15	0.0141

2. Production of ester with or without the fixative for aldehyde.

i. Sugar solution: Medium. a. Hayduck's solution (2% of sugar) 500c.c.

b. a + $CaSO_3$ 20g.

If concentration of sugar be 10%, the amount of the fixative is too weak to hold aldehyde sufficiently.

a

age of culture	PH	ester % (Volatile)	ald %	remarks
1	6.2			strong flavour
3	3.8	0.0774	0.00141	thick film.
6	3.8	0.1162	0.00253	〃
9	3.8	0.0651	0.00148	〃
12	3.8	0.0827	0.00077	〃

b

age of culture	PH	ester % (Volatie)	ald. %	alc. %	remarks
1	5.6				
3	5.6	0.0343	0.00933		
6	5.0	0.0440	0.04327	0.0948	thin film
9	5.0	0.0475	0.07490		no flavour
12	5.0	0.0580	0.10099	0.1505	slight flavour.

ii. Alcohol solution: Medium. a′ modified Hayduck's solution (Alc. 5% instead of sugar) 300c.c.

b′ a′ + $CaSO_3$ 20g.

a′

age of culture	PH	ester %	ald. %	remarks
1	6.2			strong flavour
13	3.8	0.1267	0.00933	thick film
23	3.8	0.2904	0.00594	〃
30	4.0	0.1249	—	〃

b′

age of culture	PH	ester %	ald. %	remarks
1	5.6			
13	6.4	0.0440	0.04897	film
23	6.2	0.0528	0.12559	no flavour
30	5.4	0.0792	—	〃

The quantities of ester in (i b) and (ii b′) are under the limit of analytical error caused by aldehyde.

It is not due to the reversible action of alkality shown by $CaSO_3$ that no flavour of ester was recognized, because when PH value of the medium was regulated to 6.8 – 7.6 by KOH and amino acid as buffer, ester–flavour was already felt at PH. 6.4, while initial PH of the medium with $CaSO_3$ was 5.8.

3. In the case of higher alcohol.

i. n–Propyl alcohol:

Medium: Alc. 10c.c. $(NH_4)_2SO_4$ 0·5g. Hayduck's min. sol. 4c.c. water 186c.c.

microbes: yeast mud in the same size as 4 peas.

After 9 days the flavour of propyl propionate was recognized.

After 20 days: ester 0.1756% aldehyde. 0.00765%

ii. n-Butyl alcohol: medium. 3% alcohol solution.
microbes, the same as above.

After 20 days slight ester-flavour was felt.

ON THE ORIGIN OF ALDEHYDES IN FERMENTATION PRODUCTS, I. (ACETALDEHYDE)

By MASAKAZU YAMADA

(Received Feb. 26 th., 1927)

The important rôle of acetaldehyde in saké-brewing has precisely been investigated[1] and now it is a question to determine its origin, cause of occurrence and destiny for the practical use. Many researches for the source of acetaldehyde as a by-product in alcoholic fermentation have been made[2] and it is eventually supposed that the aldehyde is not formed as an inter mediate product in the fermentation of sugar, but may arise from the secondary oxidation of ethylalcohol, although no conclusive evidence has yet been obtained.

On the one hand in many experiments for aldehyde production by several microbes[3] generally the fixative was used and so their results can not be applied directly for the case of natural sugar fermentation. Therefore, the author observed the aldehyde formation by microbes and its optimum conditions in two cases, viz., the aldehyde production in acompaniment to the fermentation of sugar and that from the oxidation of alcohol, established their relation, which had not yet been attempted and confirmed exactly that most part of acetaldehyde produced as a by-product in alcoholic fermention of sugar originated from secondary oxidation of ethyl-alcohol. The important factors that influence

(1) M. Yamada: J. Agri. Chem. Soc. Jap. **1**, 109—110, 1924.

(2) A. Trillat: Compt. rend. Acad. **146**, 645—47, 1908; A. Trillat and Sauton: Ibid. **146**, 996—9, 1908; **147**, 77—80 1908; E. Buchner, U. Langheld, S. Skraup: Ber. **47**, 2550 1914; S. Kostytschew: Ibid. **45**, 1289—93, 1912; S. Kostytschew; Z. Physiol. Ch. **79**, 130—145, 1912; C. Neuberg u. J. Kerb: Bioch. Z. **43**, 494—99, 1812; **58**, 158—70, 1913 **64**, 251. 1914; J. Laborde: Ann. Jnst. Past. **31**, 215, 1917.

(3) A. Harden: J. Ch. Soc. **79**, 610, 1901; E. R. Harding and Z. Ostenberg: J. Infect. Dis. **11**, 109, 1912; G. G. de Bord; J. Bact. **2**, 309, 1917; C. Cohen: Bioch. Z. **112**, 139—43, 1920; W. H. Peterson and E. B. Fred: J. Biol. Ch. **44**, 29—46, 1920; C. Neubery u. C. Cohen: Bioch. Z. **122**, 204—44, 1921.

the formation of acetaldehyde are the kind and quantity of microbes, temperature of culturing, sugar-concentration or alcohol-concentration produced, age of culture and aeration.

Experimental

I. Acetaldehyde production in accordance with alcoholic fermenation of sugar.

Aldehyde was estimated by Ripper's method.

1. With several microbes: The figure is g. of ald. produced per 100c.c. of koji extract culture (10° Balling at 25° C), ( ) indicates days of culture, analysed during which the fermentation appeared to end.

Willia anomala var Saké IV.	0.07235 (12)	Monilia candida	0.02610 (19)
Zygosacch. soja A	0.02020 (12)	Schizosacch. pambe	0.01746 (12)
Sacch. saké (94 kinds)	0.00136-0.02386 (7)	Mycoderma A	0.01482 (12)
Oidium G	0.01965 (14)	Mucor mucedo	0.01002 (12)
Rasse XII.	0.00695 (7)	Beer yeast Saaz	0.00635 (7)
Wine yeast	0.00484 (7)	Mucor amylomyces	0.00473 (14)
Aspergillus oryzae	0.00272 (7)	Pichia VI.	0.00188 (12)
Chalara mycoderma	0.00154 (12)		

2. Optimum temperature for aldehyde formation: 25—30°C (by 5 kinds of saké yeast)

3. Concentration of sugar: Medium: Kojiextract (at 25°C)
Microbes: Saké yeast Institute of brewing No.1 and C_1 C_4, Willia anomala var saké I (W_1)

g. of ald. and alc. % formed per 100c.c. of koji extract are following :-

		No. 1		C_1		C_4		W_1	
		Ald. g	Alc.%	Ald.	Alc.%	Ald. g	Alc.%	Ald.g	Alc. %
5°Balling	after 9days	0.00730	1.85	0.00621	2.25	0.01644	2.2	—	—
15°B	after 10days	0.01449	7.0	0.02227	—	0.04772	6.4	—	—
20°B	after 12days	0.01059	8.2	0.01882	8.8	0.04018	8.7	0.00771	—
25°B	after 9days	0.00317	10.3	0.00950	10.6	0.00574	11.25	—	—
30°B	after 13days	0.00389	13.5	0.00646	—	0.01096	13.5	0.01093	5.5

15-20% of sugar is thus optimum. The care must be taken so that the concentration of alcohol produced coincides well with the optimum concentration in the case of oxidation of alcohol.

4. Addition of mineral salts (K_2HPO_4, $MgSO_4$, $CaCO_3$, NaCl and K_2SO_4) to koji extract or the substitution of nitrogenous constituent by glycine, alanine(4), leucine, peptone,$(NH_4)_2SO_4$ and$(NH_4)_2HPO_4$ for asparagine in Hayduck's solution of controling of PH value from 2.6 to 5.8 by succinic or lactic acid exterted no special influence on the yield of acetaldehyde. Glucose, fructose, galactose mannose and glycerine instead of cane sugar gave the same as above.

5. Periods of culture: Medium: 1L. of koji extract 15°B at 25°C

(4) O. E. Ashdown and J. T. Hewitt: J. Ch. Soc. London. 97, 1636—48, 1910.

Microbes : saké yeast No. 1, C_1, C_4 and Willia(W_1)

G. of aldehyde and alcohol % produced per 100c.c. of culture are as follows :–

× Gas evolution ended

age of culture (days)	No. 1 Alc.%	Ald. g	W_1 Alc.%	Ald. g
2	3 3	0.00256	0	0.00107
5	6.1	0.00289	1.75	0.00127
8	×6.6	0.00574	3.80	0.00265
10	6.8	0.00596	4.53	0.00658
12	6.5	0.00810	×4.40	0.01809
15	6.4	0.01045	5.40	0.03567
21	6.3	0.02080	5.10	0.04352
30	5.9	0.02170	5.09	0.03617
40	5.6	0.02261	4.60	0.03014
50	5.1	0.01781	4.80	0.01302

age of culture	C_1 Ald. g	C_4 Ald. g
7	×0.00882	×0.01336
12	0.01124	0.02557
22	0.01815	0.04794
30	0.02009	0.03774
49	0.02851	0.03441

This experiment shows the most important fact, namely, in general the aldehyde is formed only in a minute quantity at the close of fermentation and then gradually increases. The maximum yield reaches after 30—40 days.

6. Aeration : There is some production of acetaldehyde, even if no aeration be taken place. But in vacuum or when oxygen was removed by alkaline pyrogallol solution no formation was observed.

II. Acetaldehyde production according to oxidation of ethyl alcohol by microbes.

1. Microbes :–

Medium : a. Alcohol 3c.c. (Alc. 96.2% Ald. 0.004206%), asparagine 0.125g, Hayduck's mineral solution 1c.c. watsr 46c.c. (Named modified Hayduck's solution)

b. $(NH_4)_2SO_4$ 0.25g. instead of asparagine in a.

c. b + $CaSO_3$ 1g. Each two platinum ears were ionoculate.d

The figures indicate the increase of aldehyde per 100c.c. of culture after 31 days at 25°C.

Microbes	a Rimini's test	aldehyde	b Rimini's test	aldehyde	c Riminis test	aldehyde
Monilia candida	++	0.04292	+	0.01893	++	0.03830
Rhizopus delemar	—	0.00150	±	0.00383	±	0.00533
Mucor mucedo	—	0.00091		—	+	0.05261
Mycoderma A	—	0.00150	+	0.02101	+++	0.07457
Zygosacch. Soja A	+++	0.04700	±	0.00338	+++	0.01044
Wine yeast	+	0.00500	±	0.00649	+	0.03086
Beer yeast Saaz	+	0.01317	±	0.00268	++	0.02511
Rasse XII	+	0.00908	+	0.00908	+	0.03378
Mucor amylomyces	+	0.00584	—	0.00286	++	0.02308
Chalara mycoderma	—	0.00036	—	0.00144	+	0.09106

Pichia	—	+	+	0.00845	卄	0.07584
Red torula	—	0	±	0.00390	卄	0.00482

2. Identification of aldehyde.

i. 3 L. of koji extract (10°B) culture of Willia IV. ii. 3 L. of the same as b in 1 inoculated with Willia IV. iii. Alc. 180c.c., $(NH_4)_2SO_4$ 7.5g., $(NH_4)_2HPO_3$ 7.5g., Hayduck min. sol. 30 c.c., water 2850c.c. inoculated with saké yeast.

	g. of ald. formed per 100c.c. after 12 days at 25°C	p-nitrophenyl hydrazone M.P	p-nitrophenyl hydrazone N (found)	N (calculated for $C_8H_9N_3O_2$)
i	——	128.5°C	23.71%	23.46
ii	0.01569	126°	23.43	〃
iii	0.01688	126°	23.12〃	〃

3. Glycine, alanine, leucine, glutamic acid, peptone and $(NH_4)_2HPO_4$ in place of asparagin in the modified alcoholic Hayduck's solution or addition of methylene-blue or $ZnCl_2$ showed no effect on the yield of aldehyde.

4. Concentration of alcohol.

Medium: 100c.c. of modified alcoholic Hayduck's solution. Yeast: Each 4 platinum ears.

Natural state

Alc. c.c.	Saké yeast (after 31days) g. of ald. produced	Willia IV.(after 15days) g. of ald. produced
1	0.00390	0.00185
2	0.00760	0.00297
3	0.01078	0.00507
4	0.01102	0.00839
6	0.01511	0.04435
8	0.03676	0.02166
10	0.02355	0.01730
12	0.00853	0.00683
14	0.00343	0.00124
16	0.00215	—
*20	0.00568	—
*30	0.00217	—
*40	0.00003	—

× Fixation

Alc. c.c.	$CaSO_3$ added	Saké yeast (after 41 days) ald. g. per 100.cc.	Willia I (after 42 days) ald. g. per 100c.c.
0.1	1g.	0.00256	—
0.2	1	0.00186	—
0.3	1	0	—
0.4	1	0.01165	—
0.5	1.5	0.04426	0.06838
1	3	0.05201	0.12040
2	4	0.03641	0.15047
3	4	0.02740	0.17075

5	5	0.02013	0.17262
8	3	0.00984	0.09592
10	3	0.00560	0.03239
12	3	0.00330	0.00347
15	3	0.00765	0.00202

× Amm. sulphate 0.25g. instead of asparagine.

* Yeast mud was added in the same size as a pea.

Optimum concentration is 6–8% in the natural state and 3–5% in the fixation.

III. Comparison of aldehyde production in both sugar fermentation and oxidation of alcohol when the fixative is used.

Medium: a. 800c.c. of koji ex. 15°B+50g. of $CaSO_3$ + saké yeast 4 platinum ears.

b. alcohol 50c.c. water 950c.c. yeast mud, 10g.

G. of aldehyde produced per 100c.c. of culture is as follows :–

after 25 hours	0.14195	after 25 hours	0.01166
〃 45 〃	0.20900	〃 3 days	0.01432
〃 94 〃	0.25622	〃 4 〃	0.01777
〃 7 days	0.22155	〃 6 〃	0.02414
〃 11 〃	0.22692	〃 13 〃	0.05512
〃 26 〃	0.28508	〃 20 〃	0.04656
〃 45 〃	0.41180	〃 39 〃	0.04303

If all the aldehyde in a 25 hour old culture in the series came from alcohol, it must be concluded that alcohol in nascent state is quite readily oxidized. But that would not be the case, and perhaps the aldehyde would have been arisen directly from the sugar as an intermediate procuct to alcohol as Neuberg's theory supposes. Whether some part of the minute quantity of aldehyde generally produced during the fermentation stage comes from sugar or not would not be decided until new method of detection for alcohol and aldehye is deviced and makes the relation of their fluctuation clear.

ON THE ORIGIN OF ALDEHYDES IN FERMENTATION PRODUCTS II.

OXIDATION OF ALCOHOLS BY MICROBES.

By Masakazu Yamada.

(Received Feb. 26th., 1927)

The existence of isobutyl-, isovaler- and other aldehydes in fermentation

products has often been reported,[1] and the aldehydes except furfurol like substance have been supposed to come from amino-acids as the hypothetic intermediate products in the so-called amino-acid fermentaion.[2]

Yet, nobody has actually isolated any aldehydes in the middle stage from amino-acid to alcohol.

On the one hand, with regards to the oxidation of alcohols by microbes there have been many researches concerning mannit, sorbit or other polyvalent alcohols,[3] but for the monovalent alcohols only a few works have been carried out.

Seifert proved that acetic acid bacteria could oxidize alcohols into the corresponding acids.[4] C. Neuberg and F. F. Nord established by the use of that the intermediate product from alcohol to acid in acetic acid fermentation was acetaldehyde.[5] Further,Trillat and Sauton said that the aldehyde formation from alcohol by yeasts was specific for ethylalcohol.[6]

The author attempted to oxidize alcohols hy two kinds of yeast and obtained propyl-, n-butyl- isobutyl-, isovaler- aldehyde from corresponding alcohols and acetone from isopropy alcohol. In order to obtain a sufficient quantity of these aldehydes or ketone from derivatives, the fixative was used. Only propion aldehyde and acetone may be obtained in a quantity without it.

In the first place, it was found that the difficulty of oxidation seems to be conversely proportional to the solubility of alcohol in water. This fact may explain an origin of aldehydes in fermentation products.

In the next place, it was observed that sake yeast could oxidize ethylalcohol as far as into acetic acid; this fact foretells the destiny of acetaldehyde and also explains a origin of acetic acid in fermentation products. Lastly, the author proved that acetic acid bacteria were capable of oxidizing propyl alcohol into propionic acid and that the inertmediate product was propyl aldehyde.

Experimental

I. Aldehyde, ketone production in modified Hayduck's solution.

Alcohol was used instead of sugar in Hayduck's solution.

---

(1) Ordonneau: Z. Spirit. Ind. **11**, 183, 1888; F, Ehrlich: Ber. **40**, 1027—47, 1907; S. Kodama: J. Ch. Soc. Japan. **43**, 956—81; 948—56, 1922; T. Takahashi: J. Agri. Japan. **65**, 1904; R. Mitsuda: J: Ch. Soc. Japan, **30**, 335—48, 1909; T. Taira: Report. Depart. Ind, Formosa, **8**, 1—7, 1925; K. Nishizaki: J. Pharm. Soc. Jap. **285**, 1029, 1905,

(2) O. E. Ashdown and J. T. Hewitt: J. Ch. Soc. London **97**, 1636—48. 1910.

(3) G. Bertrand: Compt. rend. Acad. **122**, 900, 1896; **126**, 762, 842, 984, 1897, **127**, 124, 728, 1898; Kling: Ibid. **128**, 244; **12**. 1899, Vincet u, Delachnel: Ber. **32**, 541. 1899 Boutroux: Ann. Inst. Past. **2**, 308, 1887; Alsberg: J. Biol. Ch. **9**, 1, 1911.

(4) C. **3**, 337, 897;

(5) C, Neuberg. u. F. F. Nord: Bio. Ch. Z: **96**; 133, 1919.

(6) A. Trillet et Sauton: Compt. rend. Acad. **147**, 77—80, 1908.

It was tested previously that no aldehyde was produced from asparagine by microbes, in absence of sugar.

Character of alcohols used :

	Manufacturer	aldehyde contents
n-propylalcohol	Merck	0.00764%
n-butyl 〃	〃	0
iso-butyl 〃	〃 B. P.108°	0.00482〃
isoamyl 〃	Prepared by author B. P. 128—130°	0.02267〃

Aldehyde production :-

Yeast was added in mud of the same size as a pea.

Aldehyde was estimated according to Ripper's method.

Yeast	age of culture	alcohol	total volume of culture	$CaSO_3$ g.	G. of aldehyde increased per100c.c.	remarks
sake yeast	30 days	methyl 3c.c.	50c.c.	0	0	Rimini's test-
〃	30	〃	〃	1	0	〃
〃	38	n-propyl 3	〃	0	0.01017	
〃	34	〃	〃	1	0.02484	
〃	38	n-butyl 3	〃	0	0.00347	
〃	34	〃	〃	1	0.00509	
〃	33	iso-butyl 3	〃	0	0.00143	$(NH_4)_2SO_4$ instead of asp.
〃	34	iso-amyl 3	〃	1	0.00688	〃
〃	31	iso-propyl 2	〃	1	0	Rothera's test ++
〃	31	glycerine 3	〃	0	0	
〃	31	〃 6	100	2	0.00336	
〃	31	Mannit 3	50	0	0.00140	
	31	〃 6	100	2	0.00131	
wilia I	31	Methyl 3	50	1	0	Riminis teat —
〃IV	30	n-propyl 5	100	0	0.00252	
〃 I	31	〃 3	50	1	0.00346	
〃	33	n-butyl 3	〃	0	0	
〃	31	〃 1	〃	1	0.00167	$(NH_4)_2SO_4$ instead of asp.
〃	33	iso-butyl 3	〃	0	0.00111	
〃IV	30	iso-amyl 5	100	0	0.00094	
〃 I	31	〃 1	50	1	0.00369	

II. Identification of oxidation products.

Aldehyde and ketone production in dilute alcoholic solution with the fixative by saké yeast. (at 25°C)

	alcohol c.c.	Ca SO_3	Yeast mud	total volume of culture	g. of aldhyde produced per100c c.	age of culture
1. n-propyl alcohol	40	20g.	23g.	2000c.c.	0.01923	14days
2. n-butyl 〃	30	〃	34	1500	0.02320	30
3. iso-butyl 〃	20	〃	40	1500	0.00437	27
4. iso-amyl 〃	10	〃	11.5	1500	0.01095	36
5. iso-propyl 〃	15	15	46	1000	Rothera's test++	10

Character of p-nitrophenylhydrazones prepared.

M. P.	Subst. g.	Nc.c.	P.m.m.	T	N.found	Calculated for
1. 122°	0.1183	22.7	759.2	25°	21.43%	21.76 ($C_9H_{11}N_3O_2$)
2. 88–90	0.1075	19.9	755.7	25	20.57	20.30 ($C_{10}H_{13}N_3O_2$)
3. 126	0.0526	9.7	759.0	25	20.59	20.30 ($C_{10}H_{13}N_3O_2$)
4. 110	0.0846	14.4	758.7	23	19.20	19.01 ($C_{11}H_{15}N_3O_2$)
5. 149	0.1008	18.8	764.8	18	21.68	21.76 ($C_9H_{11}N_3O_2$)

III. Acetic acid formation from ethyl–alcohol by saké yeast.

Medium : ethyl–alcohol 240c.c. (alc. 96.2% ald. 0.002074%) water 2820c.c. yeast mud 57g.

Aldehyde and total acid after 66 days at 25°C was each 0.02038g. and 0.048g. per 100c.c. of culture.

Silver content of Ag–salt prepared from the distillate :–

Subst.	AgCl	Ag. found	Ag. calculated for $C_2H_3O_2Ag$.
0.2062g.	0.1748g.	63.78%	64.64%

IV. Oxidation of propyl alcohol by Bac. xylinum at 20°C.

Medium : a. propyl alcohol 30c.c., $(NH_4)_2HPO_4$ 0.5g. KCl 0.1g water 970c.c.

b. a + $CaSO_3$ 20g.

Microbes : Bacteria–mud developed in 1 L. of koji extract.

(a.) Total acid after 35 days 0.2738g. per. 100c.c.

Silver content of Ag–salt prepared from the destillate.

Subst.	AgCl	Ag found	Ag calculated for $C_3H_5O_2Ag$
0.3282g.	0.2578g.	59.11%	56.62%

(b.) Aldehyde after 32 days 0.07948g. per 100c.c.

Character of p–nitrophenylhydrazone prepared.

M.P.	Subst.	N		N.found	N.Calc. for $C_9H_{11}N_3O_2$
1210	0.1068g	20.9c.c.	(25°C 756.2 mm.)	21 76%	21.76%.

ON THE PRODUCTION OF ACETOIN AND 2.3-BUTYLENGLYCOL BY MICROBES AND THEIR DISTRIBUTION IN FERMENTATION PRODUCTS.

By Masakazu Yamada and Kanroku Kurono.

(Received Apr. 22nd., 1927.)

It has often been reported that the reducing substance in vinegars is

acetyl methyl–carbinol.[1]

On the other hand, the production of acetoïn and its related compound, 2.3– butylenglycol by microbes, especially by several bacteria has repeatedly been described under the name of butylen glycol–fermentation in the culture media containing glucose, fructose, mannit,glycerine or Ca–lactate as a carbon source.[2] As to the production of both compounds by yeast Kluyver, Donker and Visser't Hooft confirmed that the glycol was formed everytime while acetoïn rarely in the sugar solution.[3]

The mechanism of their production is not yet accurately known except the explanation put forward by C. Neuberg and their associates. They say that acetaldehyde which is formed in nascent state as an intermediate product in the fermentation of the sugar undergoes an acyloin like conjugation with acetaldehyde, when the latter is added to the fermented liquid, by aid of carboligase and the acetoin thus formed, is reduced into butylenglycol phytochemically.[4] But Elion obtained acetoïn from the dilute ethylalcohol or acetaldehyde solution by the yeast.[5]

Lately T. Taira reported on the distribution of the butylenglycol in Japanese fermentation products, without referring to acetoïn. Therefore the production of these two compounds by microbes and their distribution in main fermentation products have been newly tested.

The results are as follows :–

1. Bacteria produced a considerable quantity of both compounds.

2. Yeasts and a fungus produced a little of both except Pichia and Chalara.

3. The butylenglycol existed in saké(fresh and old), putrid saké, shōyu, tamari–shōyu, wine, beer and vinegar, that is to say, in almost all kinds of fermentation products ; it may be regarded therefore as an ordinary component of all fermentation products.

4. Acetoïn was found in putrid saké, shōyu, tamari–shōyu and vinegar.

5. Acetoïn may be taken as a component which distinguishes the imitated vinegar from the fermented one, for it was not detected in the former.

6. Also the detection of acetoïn may be used to distinguish saké and putrid saké while it must be careful that a minute quantity was found in

---

(1) C. A. Browne : J. Pastureau :
Farnsteiner :

(2) A. Harden. u. G. S. Walpole : Proc. Roy. Soc. B. 77, 399, 1906; Harden u. Norris: Ibid. 84, 492. Ruot : C. r. Acad. 157, 247–99, 1913; M. Lemoigne : Ibid. 155, 792–95: 157, 653–55; 177, 652–54; C–r. Soc. Biol. 82, 984, 83; 336–8; 88,467.

(3) Bioch. Z. 161, 361, 1925.

(4) C. Neuberg u. E. Rein forth : Bioch. Z. 143, 553, 1923.

(5) Elion : Ibid. 40–44,

"moto" – culture of saké yeast in saké brewing and koji extract culture fermented by saké yeast.

7. There was no production of both compounds in the dilute alcohol solution cultivated with saké yeast or acetic acid bacteria contrary to Elion's experiment.

Experimental

Medium : for yeast and fingus; 50c.c. of koji extract (10°Balling) for bacteria; 50c.c. of neutralized koji extract (10°B) to which 1.5g. of $CaCO_3$ is added.

Microbes : Each 1 platinum ear was inoculated.

Detection : By Lemoigne's method developed by Kluyver and his associates. (3) (Formation of Characteristic red crystal of Nickel dimethyl-glyoxim.)

Production or existence is shown in the following table.

O was not tested.

Microbes	Sample	Acetoïn	2.3-butylen-glycol	age of culture
B.lactis 1.	15c.c.	+++++	++	12 days
2.	〃	++	–	12
B.butyricus Hüppe	〃	+++++	+++++	12
B.subtilis	〃	+++++	+++++	12
B.pyocyaneus	〃	+++++	++++	13
B.mesentericus	〃	+++	++	13
B.coli communis	〃	+++++	+++++	13
B.proteus Vulgaris	〃	+++++	+++++	13
B.acetosum xylinum Brown	〃	+++++	+++++	13
〃 (glucose solution)	〃	+++	++	13
〃 (3% alcohol Sol.)	〃	–	0	13
Zygosacch. soja A	〃	+	++	10
Schizosacch. pombe A	〃	++	++	11
Pichia IV	〃	–	–	11
Oidium A	〃	++	++++	11
Mucor mucedo	〃	++	+++	11
Chalara mycoderma	〃	–	+	11
Monilia candida	〃	++	+++++	11
Mycoderma A	〃	+	++	12
Willia anomala var saké I	〃	+	++	12
koji extract (control)	〃	–	–	0

Subst.	Vol	extractive agent	Acetoïn	Butylen-glycol
saké (fresh)	15c.c.	—	–	++
〃 〃	650	ether	–	0
〃 〃	〃	$CHCl_3$	–	0
〃 (old)	〃	ether	–	++

Shōyu	150	$CHCl_3$	+ +	+ + + +
Tamari-shōyu	100	$CHCl_3$	+ +	+ +
wine	15	–	0	+ +
beer (Yebisu)	500	$CHCl_3$	–	+
〃 〃	15	–	–	–
vinegar	15	+	+ +	+ + +
moto	15	–	–	+
〃	900	$CHCl_3$	+	0
putrid saké	15	–	+	+ +
Koji ex. fermented by saké yeast after 6 days	15	–	–	±
〃	500	$CHCl_3$	+ +	+
Koji extract(not fermented)	2000	ether	–	–
5% alcohol solution with saké yeast	1400	ether	–	–

昭和二年六月七日印刷
昭和二年六月十日發行

東京帝國大學農學部內
發行兼編輯者 松山芳彥

東京帝國大學農學部內、日本農藝化學會
印刷者 河村秀兼

東京帝國大學農學部內
印刷所 農藝化學敎室印刷所